

ModDetect™ IHC Protocol

I. Reagents Required

Product	Preparation	Suggested Product(s)
Phosphate Buffered Saline (PBS)	Use 10X PBS, pH 7.2 (0.2 M Potassium Phosphate, 1.5 NaCl) Dilute appropriate volume to 1X with deionized water.	MB-008
Xylene		
95% and 100% Ethanol		
Triton-X 100		
UltraPur Sterile Water		MB-009-1000
Antibody Dilution Buffer	Prepare 100 mL of PBS, supplemented with 1 mL of normal serum of same species as host for the secondary antibody.	
30% Hydrogen Peroxide Solution		KHJ001
Peroxidase Secondary Antibody	1:500	
Streptavidin Peroxidase Conjugated	1:500	
DAB Substrate or TMB Membrane Peroxidase Substrate	(For stable brown or blue staining, respectively.)	DAB-10; TMBM-100
Polymount Mounting Media		KHH001
0.1 N Sodium Hydroxide Solution	(For pH adjustment)	
0.1 N Hydrochloric Acid Solution	(For pH adjustment)	
0.2 M Hydrochloric Acid Solution 22 mL		
Methanol Industrial Methylated Spirits (IMS) or Methanol		
Tri-Sodium Citrate 2.94 g		

II. Procedure for Paraffin Sections

1. Deparaffinize sections in xylene 2 times for 5 minutes.
2. Hydrate with 100% ethanol 2 times for 3 minutes.
3. Hydrate with 95% ethanol for 1 minute.
4. Rinse in UltraPure sterile water.

III. Heat-induced Epitope Retrieval Procedure

1. De-wax and rehydrate the paraffin sections by placing them in 3 changes of xylene for 3 minutes each, followed by 3 changes of IMS or methanol for 3 minutes each, followed by cold running tap water. Keep them in the tap water until the microwave antigen retrieval solution has been prepared. At no time from this point onward should the slides be allowed to dry out!
2. Add the tri-sodium citrate, hydrochloric acid, and UltraPure Water together in a 1L beaker/conical flask. Use a magnetic stirrer to ensure that all reagents are properly dissolved. Adjust to pH 6.0 using the sodium hydroxide and hydrochloric acid solutions. Add this solution to the microwaveable vessel.
3. Remove the slides from the tap water and place them in the microwaveable vessel. Place the vessel inside the microwave. If domestic, set to full power and wait until the solution comes to the boil. Boil for 15 minutes from this point. If scientific, program so that antigens are retrieved for 15 minutes once the temperature has reached 98°C.

- When 15 minutes has elapsed, remove the vessel and run cold tap water into it for 10 minutes.
- Permeabilization is suggested prior to staining to access intracellular targets. We recommend 0.1%–0.4% Triton X-100 to permeabilize cells, however, depending on intracellular localization of target and cell or tissue type other methods for permeabilization should be explored to optimize results.
- Continue with the immunohistochemical staining protocol.

IV. Procedure for Immunoenzyme Staining

Note: Avoid Mouse-on-Mouse interference, contact us for recommendations.

- Follow procedure for pretreatment as required.
- Rinse sections in PBS 2 times for 2 minutes.
- Incubate sections in normal serum block with the same species as the secondary antibody (e.g. Normal Goat Serum (NGS) if secondary antibody is goat host).
Note: Blocking endogenous biotin may be needed.
- Incubate sections in primary antibody at appropriate dilution (1:200–1:2,000 recommended) in dilution buffer for 1 hour at room temperature or overnight.
Note: Do not rinse sections between serum block and primary antibody incubation.
- Rinse in PBS buffer 3 times for 2 minutes.
- Incubate sections in 1% hydrogen peroxidase in PBS for 10 minutes at room temperature.
- Rinse in PBS buffer 3 times for 2 minutes.
- Incubate sections in secondary reagent in PBS buffer for 30 minutes at room temperature.
- Rinse in PBS buffer 3 times for 2 minutes.
- Incubate sections in streptavidin peroxidase in PBS buffer for 30 minutes at room temperature.
- Rinse in PBS buffer 3 times for 2 minutes.
- Incubate sections in peroxidase substrate solution.
- Rinse in PBS buffer 3 times for 2 minutes.
- Rinse in UltraPure water 3 times for 5 minutes.
- Dehydrate through 95% ethanol for 1 minute, 100% ethanol 2 times for 3 minutes.
- Clear in xylene 2 times for 5 minutes.
- Coverslip with mounting medium.

V. Supporting Products

Product	Item No.
Blocking Buffer for Immunohistochemistry - Serum and Azide Free	MB-071-0100
10X PBS MaxTag Histo for IHC	MB-0032
Pap Pen MaxTag Histo for IHC	KHP-001
30% (w/v) Hydrogen Peroxide Solution MaxTag Histo for IHC	KHJ001
Polymount Mounting Media MaxTag Histo for IHC	KHH-001
Ferrialdehyde Fixative 16% (w/v) Ultrapure EM Grade MaxTag Histo for IHC	KHF-001
BBS Fish Gel Concentrate	MB-065-0100
PBS Fish Gel Concentrate 10X	MB-066-0100
TBS Fish Gel Concentrate 10X	MB-067-0100
Normal Rabbit Serum	B309
Normal Goat Serum	B304

Supporting Products Available via antibodies-online

Endosome Marker Antibodies	Item No.
Anti-EEA1 (RABBIT) Antibody	ABIN521882

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Anti-RAB7B (AA 100-199) Antibody	<u>ABIN567027</u>
Anti-RAB5 Antibody	<u>ABIN361846</u>
Anti-RAB5B (C-Term) Antibody	<u>ABIN1439995</u>
Anti-RAB5C (C-Term) Antibody	<u>ABIN6254194</u>
Anti-RAB7A (C-Term) Antibody	<u>ABIN720191</u>
Anti-RAB9A (RABBIT) Antibody	<u>ABIN564041</u>

Lysosome Marker Antibodies	Item No.
Anti-Cathepsin L (AA114-288) Antibody	<u>ABIN7431587</u>
Anti-CTSA (N-Term) Antibody	<u>ABIN654433</u>
Anti-Cathepsin D (C-Term) Antibody	<u>ABIN6254162</u>
Anti-LAMP1 (AA 80-280) Antibody	<u>ABIN3016286</u>
Anti-LAMP2 (AA 191-362) Antibody	<u>ABIN7437248</u>
Anti-Cathepsin K (AA 54-317) Antibody	<u>ABIN7441856</u>
Anti-Cathepsin S (AA 115-331) Antibody	<u>ABIN7434368</u>

Cytoskeleton Marker Antibodies	Item No.
Anti-Vimentin (AA 371-466) Antibody	<u>ABIN672786</u>
Anti-Alpha Tubulin Antibody	<u>ABIN93891</u>
Anti-TUBB Antibody	<u>ABIN93914</u>
Anti-Smooth Muscle Actin (N-Term) Antibody	<u>ABIN6254917</u>
Anti-ACTC1 Antibody	<u>ABIN2855212</u>
Anti-ACTN2 Antibody	<u>ABIN2855582</u>
Anti-ACTG2 (AA 3-376) Antibody	<u>ABIN7440569</u>
Anti-Myosin (AA 1069-1331) Antibody	<u>ABIN7439273</u>
Anti-Alpha Actinin Antibody	<u>ABIN7073023</u>
Anti-Dystrophin (AA 346-635) Antibody	<u>ABIN1679546</u>
Anti-ACTN3 (N-Term) Antibody	<u>ABIN6258681</u>
Anti-Cytokeratin 8/18 Antibody	<u>ABIN285686</u>